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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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COMPOSITIONS AND METHODS FOR TREATMENT OF AUTOIMMUNE DISEASES

Field of the Invention

This invention relates to methods of treating diseases of in which the immune system is involved. In particular, this invention relates to methods of treating autoimmune diseases.

Background of the invention

Autoimmune diseases

One of the most intriguing characteristics of the immune system is its unlimited specificity. When threatened by potentially dangerous foreign substances (antigens), including pathogens, the immune system mounts a tailor-made response. This tailor-made response is provided by the immune systems antigen specific T and B lymphocytes. The virtually unlimited repertoire provided by these immune cell calls for a tight regulatory system preventing the recognition of our own (self) antigens. For years it was thought that the immune system was able to discriminate between self and non-self. However, with the growing knowledge of immunology, this theory has become more and more unsatisfactory. The self/non-self paradigm does not explain why perfectly healthy individuals can have circulating autoreactive T and B cells without any symptoms of autoimmune diseases.

Recently, a new concept providing more satisfactory explanations for the lack of autoimmune reactions in healthy individuals was developed. In this new hypothesis, the decision whether the immune system is activated does not solely depend on the recognition of an antigen as foreign, but also on the immune systems judgment whether it imposes danger to the integrity of the individual. The immune response must be considered as an outcome of a complex interaction between the lymphocyte and the antigen presenting cell (APC) in the context of cognate co-stimulatory signals and the local cytokine microenvironment in which the recognition of the specific antigen takes place. This new view on the immune system does not only provide explanations for issues that made us doubt about the self/non-self paradigm, it also provides more insight in the mechanisms of central and peripheral tolerance.

Th1 and Th2 cells: the role of Th1 cells in autoimmune diseases

Helper T cells regulate immune responses via cytokines that they produce upon

recognition of specific antigen presented by antigen presenting cells. Individual Th cells (clones) can be distinguished on the basis of the cytokine secretion profile and hence their function (Mosmann et al., *Annual Review of Immunology* 7: 145 (1989)). In response to most antigens, Th cells produce many cytokines simultaneously (type 0 cytokine profile). However, in response to certain types of antigens the Th cell response is biased to low levels of interferon-gamma (IFN-γ)_and high levels of interleukin-4 (IL-4) and interleukin-5 (IL-5) (type 2 cytokine profile, Th2). In contrast, in response to certain other antigens, the production of cytokines of the Th cells is biased to high levels of IFN-γ and low levels of interleukin IL-4 and IL-5 (type 1 cytokine profile, Th1). There is accumulating evidence that type 1 and 2 profiles result from modulation of the local cytokine microenvironment (Trinchieri, *Immunology Today* 13: 379 (1993); Snijdewint et al., *J. Immunology* 150: 5321 (1993)). Various factors may directly act on the T cells, but they may also act indirectly by affecting antigen-presenting cells, which in turn secrete mediators that skew to Th1 or Th2 profiles.

Clearly, soluble factors secreted by antigen presenting cells during antigen-presentation are important. Antigen presenting cell-derived factors that skew T cell cytokine production towards Th1 and Th2 profiles include interleukin-12 (IL-12) and prostaglandin E2 (PGE-2). A low IL-12/PGE-2 production ratio in antigen presenting cells will results in IL-4 dominated T cell responses, whereas a high IL-12/PGE-2 production ratio will result in IFN-γ-dominated T-cell responses.

It is the current believe that many autoimmune diseases are caused by autoreactive Th1 cells. In experimental autoimmune models, the phenotype of T cells that induce disease has extensively been studied. Experimental autoimmune encephalomyelitis (EAE) is a model for multiple sclerosis. In this model that can be induced by transfer of T cells specific for central nervous system (CNS) antigens, the pathogenic T cells secrete a type 1 cytokine profile (Zamvil and Steinman, *Ann. Rev. Immunol.* 8: 579 (1990)). Likewise, in the non-obese diabetic (NOD) mouse model, transfer of T cells specific for a pancreatic autoantigen that had been differentiated in the presence of type 1 cytokines in vitro, caused disease, while the same T cells that had been differentiated in the presence of type 2 cytokines did not (Katz et al, *Science* 268: 1185 (1995)).

Evidence in humans for the mutual exclusive relationship between Th1 and Th2 response came from a recent study among Japanese school children that show a strong inverse relationship between delayed hypersensitivity responses to *M. tuberculosis* (Th1-type of response) and the presence of asthma, serum IgE levels and Th2-cytokine

profiles (Shirakawa et al., *Science* 275: 77 (1997)).

IL-12, a major regulator of type 1 T-cell cytokine responses

IL-12 is a heterodimeric glycoprotein composed of two covalently linked peptide chains, called p40 and p35 (Trinchieri *Ann. Rev. Immunol.* 13: 251 (1995)). IL-12 is mainly produced by activated monocytes and dendritic cells. IL12 can be produced by monocytes after stimulation with bacterial products such as LPS or after stimulation with activated T cells. For dendritic cells the ligation of CD40 with CD40L on the surface of activated T cells is the strongest trigger for IL-12 production. The most pronounced effect of IL-12 is the stimulation of IFN-γ by human NK cells and T cells. IL-12 exerts its effects through binding to a high affinity receptor. The functional, high-affinity IL-12 receptor (IL-12R) consist of a β1 and a β2 chain, of which only the latter is involved in signal transduction. The nucleotide and amino acid sequences of the IL-12 receptor β1 chain are disclosed in EP-A-638644. The sequences of the IL-12 receptor β2 chain are disclosed in EP-A-759466.

Immunotherapy for autoimmune diseases targeting autoreactive T cells

Currently used drugs for the treatment of autoimmune diseases are primarily directed at the treatment of symptoms. Most, if not all of these drugs are ineffective at stopping the disease process, need to be administered chronically and are often associated with significant side effects. This makes the presently used drugs highly unfavourable. Optimal drugs for the treatment of autoimmune diseases will be able to attenuate the autoimmune process by re-establishing the immune system's self-regulatory mechanisms that have failed and resulted in the autoimmune attack. Treatment during the early phase of the autoimmune process with such drugs have the potential to arrest the disease process.

It has been demonstrated that T cells play a central role in the auto-destructive process in autoimmune diseases such as rheumatoid arthritis (Sigall et al., *Clin. Exp. Rheum.* 6: 59 (1988)). Treatments that selectively suppress the activity of such autoreactive T cells can therefore be preferred. Such treatment could consist of the administration of an autoantigen or peptides derived thereof. This type of treatment has been very successful in the suppression of disease symptoms in various experimental autoimmune disease models in laboratory animals and it has been suggested that successful therapy is associated with the up-regulation of Th2 responses and a down-

regulation of Th1 responses. It is therefore suggested by the present inventors to combine antigen-specific therapy targeting autoreactive T cells with the modulation of the cytokine microenvironment.

Summary of the Invention

The current invention is based on the finding that Th2 cell development from naive Th cells is associated with suppression of IL-12R β2 chain expression leading to loss of IL-12 responsiveness and, consequently, the inability to promote IFN-γ production. Furthermore, the present invention is based on the finding that allergen-specific Th2 clones generated from atopic patients do not produce IFN-γ. Even upon exposure to IL-12, IFN-γ protein and mRNA expression cannot be induced in such clones. Further analyses revealed the complete lack of signalling via the IL-12R in these Th2 clones, as indicated by their inability to phosphorylate STAT4 despite the abundant presence of this selectively IL-12-induced transcription factor. FACS analysis showed normal expression of the IL-12R β1 chain. These findings strongly suggest the absence of functional β2 chains on human Th2 cells, similar to mouse Th2 cells. RNAse-protection assays with a human IL-12R β2 chain-specific DNA probe indeed indicated the absence of IL-12R β2 mRNA in activated Th2 clones.

Accordingly, the inventors propose to specifically neutralize the activity of the IL-12R β2 chain. Specific neutralization of the IL-12R β2 chain can be accomplished by a specific monoclonal antibody that binds to the IL-12R β2 chain, but does not stimulate the phosphorylation of STAT4. Such an antagonistic monoclonal antibody to the IL-12R β2 chain can be used to prevent or treat diseases in which activated type 1 T cells are involved. Such an antagonistic monoclonal antibody can be used to enhance the effect of antigen-specific therapy of autoimmune diseases targeting Th1-like autoreactive T cells.

Accordingly, it is a primary object of this invention to provide a pharmaceutical composition comprising of a therapeutically effective amount of a monoclonal antibody capable of binding to the human IL-12R β2 chain, where said binding prevents interleukin-12 from mediating a signal through said receptor resulting in the phosphorylation of STAT4, in a pharmaceutically acceptable excipient.

It is an other objective of the present invention to combine a monoclonal antibody capable of binding to the human IL-12R β2 chain, where said binding prevents interleukin-12 from mediating a signal through said receptor resulting in the

phosphorylation of STAT4, with specific autoantigens, modified autoantigens or peptide fragments thereof.

It is an other objective of the present invention to combine a monoclonal antibody capable of binding to the human IL-12R β2 chain, where said binding prevents interleukin-12 from mediating a signal through said receptor resulting in the phosphorylation of STAT4, with other therapeutic monoclonal antibodies, such as monoclonal antibodies to co-stimulatory receptors on T cells or antigen presenting cells such as CD40, CD40L, CD80 and CD86.

It is a further objective of this invention to provide a method for treating autoimmune diseases, the method comprising administering to a patient in need of such treatment a therapeutically effective amount of a monoclonal antibody capable of binding to the human IL-12R β2 chain, where said binding prevents interleukin-12 from mediating a signal through said receptor resulting in the phosphorylation of STAT4, in a pharmaceutically acceptable excipient. In particular, said administration is combined with the administration of specific autoantigens, modified autoantigens or peptide fragments thereof, or, alternatively, with the administration of other therapeutic monoclonal antibodies, such as monoclonal antibodies to co-stimulatory receptors on T cells or antigen presenting cells, including CD4, CD40, CD40L, CD80 and CD86.

Detailed description of the invention

The invention pertains to antibodies, preferably monoclonal antibodies, capable of binding to the β2 chain of the IL12 receptor. The binding should be such that phosphorylation of a Signal Transducer and Activator of Transcription (STAT), specifically STAT4, is not activated. The activation of STATs by tyrosine phosphorylation in response to external stimuli such as cytokines was described by Schindler and Darnell, *Rev. Biocehem.* 64: 621 (1995). Of the STAT molecules, STAT4 is the only one that is tyrosine phosphorylated after stimulation of T cells with interleukin 12. The molecular cloning of STAT4 base on its homology with STAT1 was described by Yamamoto et al, *Molec. Cell Biol.* 14: 4342 (1994).

The invention further pertains to antibodies, preferably monoclonal antibodies, capable of binding to the β2 chain of the IL12 receptor, especially to an epitope of IL12R β2 chain such that binding of the β2 chain to the IL12R β1 chain in prevented. Particularly preferred are antibodies that prevent dimerisation of β2 chain to β1 chain and also prevent activation of phosphorylation of STAT4.

As used herein, the term "antibody" refers to polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, single-chain antibodies, and fragments thereof such as Fab, F(ab')2, Fv, and other fragments which retain the antigen binding function of the parent antibody.

As used herein, the term "monoclonal antibody" refers to an antibody composition having a homogeneous antibody population. The term is not limited regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made. The term encompasses whole immunoglobulins as well as fragments such as Fab, F(ab')2, Fv, and others which retain the antigen binding function of the antibody. Monoclonal antibodies of any mammalian species can be used in this invention.

As used herein, the term "chimeric antibodies" means that the constant regions of an immunoglobulin are derived from human immunoglobulin sequences.

As used herein, the term "humanized antibodies" means that at least a portion of the framework regions of an immunoglobulin are derived from human immunoglobulin sequences.

As used herein, the term "single chain antibodies or ScFv" refers to antibodies prepared by determining the binding domains (both heavy and light chains) of a binding antibody, and supplying a linking moiety which permits preservation of the binding function. This forms, in essence, a radically abbreviated antibody, having only that part of the variable domain necessary for binding to the antigen. Determination and construction of single chain antibodies are described in U.S. Patent 4,946,778, incorporated herein by reference.

As used herein, the terms "CD80", "CD86", "CD40" and "CD40L" refer to human surface molecules as extensively reviewed in Van Gool et al., *Immunol. Rev.* 153: 46 (1996), incorporated herein by reference). For immunization purposes CD80, CD86, CD40 and CD40L antigen may be prepared by any technique known in the art. Antibodies human CD80, CD86, CD40 and CD40L are known in the art. The present invention also contemplates a new use for such antibodies as detailed above.

As used herein, the term "autoantigen" refers to a human protein that is recognized by autologous T cells, resulting in self-tissue destruction in autoimmune disease patients. Examples of autoantigens that are recognized by autologous T cells are myelin basic protein in multiple sclerosis; collagen type II and human cartilage glycoprotein 39 (WO 96/13517) in rheumatoid arthritis; insulin and glutamic acid

decarboxylase (diabetes); and alpha-fodrin (Sjögren's syndrome). For therapeutic use, autoantigens may be administered in there native form, modified by selected amino acid substitutions (WO 96/16085), or in peptide fragments with (Kumar et al., *Proc. Natl. Acad. Sci.* 87: 1337 (1990), both incorporated herein by reference) or without selected amino acid substitutions.

As used herein, the term "interleukin-12 receptor" refers to the human surface molecule capable of binding human interleukin-12 as reviewed above. For immunization purposes the human interleukin-10 antigen may be prepared by any technique known in the art.

As used herein, the term "antagonistic" refers to the capacity of a soluble ligand to bind to a cell surface receptor, where said binding prevents intracellular signal transduction leading to the activation of said cell surface receptor by the natural ligand for said.

The pharmaceutical compositions of this invention are administered at a concentration that is therapeutically effective to modulate the hosts immune response. To accomplish this goal, the pharmaceutical composition may be formulated using a variety of acceptable excipients known in the art. Typically, the pharmaceutical composition is administered by injection, either subcutaneous, intramuscular, intravenous or intraperitoneal. Methods to accomplish this administration are known to those of ordinary skill in the art. It may also be possible to obtain compositions which may be orally administered, or which may be capable of transmission across mucous membranes. Before administration to patients, formulants may be added to the pharmaceutical composition.

The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should be noted that these embodiments are illustrative and are not to be construed as restricting the invention in any way.

EXAMPLES

Example 1

IL-12 modulates the production of IL-4, IL-5 and IFN-γ by stimulated CD4+ T cells

Naive CD45RA $CD4^+$ T cells were isolated from the heavy fraction of PBMC in a two step protocol. First $CD4^+$ cells were isolated by incubation with CD4 specific Dynabeads followed by Detatchabead treatment, as indicated by manufacturer (Dynal,

Oslo, Norway). In the second step, UCHL-1 and HLA-DR positive cells were removed by panning, after labelling with appropriate antibodies. This procedure yielded a population of more than 98% $CD45RA^+$, $CD4^+$ T cells. These naive CD45RA $CD4^+$ T cells were stimulated in 96-well flat-bottom culture plates (Costar, Cambridge, MA) in Iscove's modified Dulbecco's medium (IMDM; Life Technologies, Paisley, UK), supplemented with 5 % pooled, C-inactivated normal human serum (CLB). To assess the direct modulatory effects of exogenous IL-12, T cells (2×10^4/well) were stimulated in the absence of accessory cells (AC) with a combination of immobilized anti-CD3 mAb (1 µg/ml), soluble anti-CD28 mAb (1 µg/ml) and with IL-2 (5 U/ml). IL-12 (200 U/ml) was added to the cultures at the start of the T cell stimulation. After 12 days of culture, resting T cells were harvested and restimulated with immobilized anti-CD3 mAb (1 µg/ml), soluble anti-CD28 mAb (1 µg/ml). Supernatants were harvested after 24 hours and analysed for the presence of cytokines by ELISA techniques as described by Van der Meide et al. (*J. Immunol. Methods* 79,293 (1985)) for IFN-γ, Van der Pauw-Kraan et al. (*Eur. Cytokine Network* 4: 343 (1993)) for IL-4 and McNamee et al. (*J. Immunol. Methods* 141: 81 (1991)) for IL-5.

In figure 1 it can be seen that addition of IL-12 during the priming of naive T cells strongly stimulates the production of the type 1 T-cell cytokine IFN-γ, but inhibits the production of the type 2 T-cell cytokines IL-4 and IL-5.

Example 2

Cloning of the human IL12R ß2 chain and expression on the surface of insect cells

The human IL12 receptor (IL12R) consist out of two chains, called ß1 and ß2. These form a heterodimer in order to act as a functional molecule on the cell-membrane. The ß2 chain is responsible for the transduction of signals into the IL12R expressing cells. The cDNA encoding the ß2 chain of the human IL12R was generated by PCR from RNA isolated from PBMC's. Briefly, the PBMC's were separated from red blood cells by gradient centrifuge using Ficoll, after which the lymphocyte fraction was stimulated for 2 - 20 h with PMA (1ng/ml) and ionomycin (1µg/ml) in IMDM/FCS at 37°C with 5% CO2. Subsequently messenger RNA was prepared from the cells. The cells were washed twice with phosphate buffered saline (PBS pH 7.4) and lysed in 5M guanidinium thiocyanate in the presence of 0.7 M 2-mercaptoethanol. The RNA was bound on a Qiagen spin column, washed according to manufactures protocol and eluted in DEPC treated water. RNA was stored in -70°C.

First strand cDNA was synthesized by incubation at 37°C for 1 hour of 1–5 μg total RNA in a 50 μl mix, consisting of 1x synthesis buffer (USB), 10mM dNTP, 5 μM random hexamers and 5 U M–MLV–reverse transciptase (USB). This was followed by a incubation of the tube at 70°C for 10 min. After cooling on ice from this mixture 2.5 μl was used as template in a PCR reaction using primers specific for respectively the IL12R ß2 chain. These primers (SEQ ID NO 1 and 2)were based on the published cDNA coding sequences for IL12R ß2 (Presky D.H. et al., *Proc. Natl. Acad. Sci. USA* 93: 14002 (1996)).

SEQ ID NO 1 5' – gcgcgaatct tgttgatggc acatactttt ag – 3'

SEQ ID NO 2 5' – gcgccccggg tcagagcatg agggagtcac acc – 3'

Both the sense and the anti–sense primers start with GCGC followed by a restriction site for cloning purpose. The sense primers carriers the ATG start codon, while the anti–sense primers contains a stop codon. The amplified cDNA will encode for the full–length IL12R ß2 chain including the naturally occurring signal peptide. To amplify the IL12R ß2 chain a standard PCR was done. The PCR mixture of 100 μl contained 1x PCR buffer, 2.5U Taq polymerase, 0.25 mM dNTPs, 25 pmole of each primer and 2.5 μl cDNA template. The mixture was run in Perkin Elmer thermocycler for 20 – 40 cycles of 1 min 95°C, 1 min 55°C, and 2 min 72° C followed by 1 step for 7 min at 72°C as extension of the PCR product. The obtained PCR product was gel purified and cloned in pCR Script using the Stratagene cloning kit. Briefly, the PCR product was incubated with plasmid together with T4 ligase and SrfI for 1h at KT, after which the entire sample was transformed in Xl1Blue E.coli cells. The cells were plated on LB plates containing 100 μg ampicillin/ml, 20 μg IPTG/ ml and 20 μg Xgal/ml. After incubation over night at 37°C putative white clones were analysed for having an insert. Clones containing inserts were analysed by cycle sequencing using M13 and M13 reverse primers. Several clones were identified containing a DNA sequence encoding for the IL12R ß2 chain. By further sequencing a correct cDNA clone encoding full–length II12R ß2 chain was found without PCR induced mutations.

To express the IL12R ß2 chain on the cell surface of Sf9 insect cells, the obtained cDNA was re–cloned in the baculovirus transfer vector pVL1392. The pVL1392 vector and the IL12R ß2 chain cDNA cloned in pCR Script were digested with EcoRI and SmaI. The IL12R ß2 chain insert and the linear pVL1392 were gel purified, after which the insert was ligated in pVL1392. The ligation mixture contained 100 ng plasmid, 100 ng insert, 1x ligase buffer and T4 DNA ligase (Promega). The ligation mixture was transformed to DH5a, and plated on LB plates containing 100 μg ampicillin/ml. After

incubation over night at 37°C the clones were screened for having the correct plasmid. A pVL1392 plasmid containing the IL12R ß2 chain insert was selected and large scale plasmid preparation was done using the midi-prep system from Qiagen.

Example 3

Baculovirus expression of human IL12R ß2 chain and generation of monoclonal antibodies

Using the transfer vector pVL1392 containing the full-length IL12R ß2 chain cDNA obtained in example 2, the sequence was recombined into the Autographa californica baculovirus (AcNPV). Briefly, using the BaculoGold system from Pharmingen each recombinant plasmid was separately cotransfected with wild-type baculoviral DNA containing a lethal deletion in a 4 to 1 ratio into Sf9 (Spodoptera frugiperda) cells. Recombinant baculovirus was plaque purified, followed by several rounds of amplification to obtain a high titer recombinant virus stock.

For the generation on monoclonal antibodies, Sf9 cells were infected with the IL12R ß2 chain carrying virus. Briefly, Sf9 cells were infected with recombinant virus at a MOI of 10. The cells were harvested after 48-72 hours of culture in TC100 FCS at 28°C under standard conditions. The cells were washed with PBS twice followed by injection intraperitoneally in female BALB/c mice (5-10 x 106 Sf9 IL12R+ cells/ mouse). At day 14, 21 and 28 the mice received a new booster injection with Sf9 IL12R+ cells. Three days after the last injection the spleen cells were isolated and used for cell fusion with SP2/0 murine myeloma cells at a ratio of 10:1 using 50% polyethylene glycol. The fused cells were re-suspended in IMDM/FCS supplemented with HAT, followed by plating on 96 wells plates. After 10 - 14 days wells containing growing hybridoma cells were screened for antibody production. The supernatants of 12 wells were pooled and used in a FACS analysis using human type 1 T-cell clones. Briefly, T cells (0.1 - 0.2 x 10^6/sample) were incubated for 20 min at 4°C with the pooled supernatants. After washing with FACS buffer (PBS pH7.4 1%BSA 0.1% NaN_3), the cells were incubated for another 20 min at 4°C with goat anti-mouse antibodies conjugated to fluorescein isothiocyanate (FITC). The cells were washed with FACS buffer and finally suspended in FACS buffer containing 0.5% paraformaldehyde and analysed with a FACScan flow cytometer (Becton Dickinson). Subsequently, the supernatants of positive pools were tested in the same assay individually. Positive hybridoma cells were cloned at least three times by limiting dilution in IMDM/FCS IL-

6. The selected positive hybridoma clones were subsequently further characterized and selected by their ability to block the signal transduction via the IL12R expressed on activated type-1 T cells.

Example 4

Characterization of anti-IL-12R β2 monoclonal antibodies

Resting CD4+ PBT cells are stimulated in 96-well flat-bottom culture plates (Costar, Cambridge, MA) in Iscove's modified Dulbecco's medium (IMDM; Life Technologies, Paisley, UK), supplemented with 5 % pooled, C-inactivated normal human serum (CLB) with IL-2 (10 U/ml). To assess the direct modulatory effects of exogenous IL-12, T cells (105/well) are stimulated in the absence of accessory cells (AC) with a combination of immobilized anti-CD3 mAb (1 μg/ml) and soluble anti-CD28 mAb (1 μg/ml). Increasing concentrations of IL-12 are added with or without blocking anti-IL12R ß2 monoclonal antibodies to the cultures at the start of the T cell stimulation. PBT cell supernatants are collected 42 h and IFN-γ levels are analysed. It is demonstrated that specific monoclonal antibodies are able to prevent IL-12 induced IFN-g production.

For analysis of IL-12-induced tyrosine phosphorylation of STAT4, $5x10^6$ TLC cells are or are not exposed to IL-12 (100 U/ml) for 20 min. in the absence or presence of anti-IL-12R monoclonal antibodies, washed twice with ice-cold PBS and lysed in 250 μl of immunoprecipitation buffer (IPB) [10 mM Tris/HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 % Nonidet P-40, 0.25 % sodium deoxycholate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM PMSF, 1mM sodium orthovanadate and 10 mM NaF]. Lysates are precleared by three incubations with 50 μl of a 10% (v/v) suspension of non-immune mouse Ig-coated protein A-CL4B Sepharose beads (Pharmacia, Uppsala, Sweden), and once with uncoated protein A-Sepharose beads. Precleared lysates are then incubated with anti-STAT4 (C20, Santa Cruz) for 30 min followed by protein A-Sepharose beads for 2 h. After washing in IPB, the STAT4 immunoprecipitates are resuspended in sample buffer, separated by SDS-PAGE under reducing condition, and transferred to Hybond C nitrocellulose membrane (Amersham Co., Aylesbury, UK), employing a semidry electroblotting chamber (Multiphore II, Pharmacia). Blots are saturated with blocking buffer [50 mM Tris, 150 mM NaCl (pH 7.5) containing 0.2 % Tween and 1% BSA] and incubated with horseradish peroxidase-labelled anti-phosphotyrosine (RC20; Signal Transduction Laboratories, Lexington, KY)

for 1 h. Phosphorylated tyrosine residues are visualized using enhanced chemiluminescence (ECL, Amersham). For detection of STAT4 proteins on the same blots, deprobing of the blots is performed according to the manufacturer's instructions. Blots are then incubated with anti-STAT4 (C20, Santa Cruz Biotechnology) for 1 h, washed, incubated for 1 h with horseradish peroxidase-labelled horse anti-rabbit Ig (CLB), and visualized as described above. It is demonstrated that the specific anti-IL12R ß2 monoclonal antibodies can prevent the phosphorylation of STAT4 and thus are potent inhibitors of the signal transduction cascade in lymphocytes leading to a strong type-1 pro-inflammatory cytokine production.

Example 5

Modulation of autoreactive T cells by anti-IL12R ß2 chain monoclonal antibodies and specific autoantigen

Resting PBT cells are cultured in 96-well flat-bottom culture plates (Costar, Cambridge, MA) in Iscove's modified Dulbecco's medium (IMDM; Life Technologies, Paisley, UK), supplemented with 5 % pooled, C-inactivated normal human serum (CLB) in the presence of autologous dendritic cells pulsed with specific autoantigen peptide and in the presence or absence of specific anti-IL12 R ß2 monoclonal antibodies. After 6 to 7 days rIL-2 (10 U/ml) is added and the specific T cells were allowed to expand for an additional 6 to 7 days. After this culture period the specific T cells were restimulated with the specific autoantigen peptide and the culture supernatants were analysed for the production of the type-1 cytokine IFN-γ_and the type-2 cytokine IL-4. Autoantigen-specific T cells primed in the presence of specific anti-IL12 R ß2 monoclonal antibodies produced less IFN-γ_and more IL-4 than T cells stimulated without the specific anti-IL12 R ß2 monoclonal antibodies.

Claims

1. A monoclonal antibody that can bind to the IL12R ß2 chain expressed on the cell surface of human T lymphocytes, where said binding prevents IL12R ß2 chain–mediated STAT4 phosphorylation.

2. A monoclonal antibody that can bind to the IL12R ß2 chain expressed on the cell surface of human T lymphocytes, where said binding prevents the IL12R ß2 chain from the formation of a complex with other membrane–associated proteins.

3. A monoclonal antibody that can bind to the IL12R ß2 chain expressed on the cell surface of human T lymphocytes, where said binding prevents the IL12R ß2 chain from dimerisation to the IL12R ß1 chain.

4. A combination of a monoclonal antibody of any one of claims 1–3 or part thereof, and an autoantigen, peptide fragments of an autoantigen or a modified form thereof.

5. A combination according to claim 4, wherein said autoantigen is selected from myelin basic protein, collagen type II, human cartilage glycoprotein 39, heat shock proteins, insulin, glutamate decarboxylase and α–fodrin.

6. A combination of a monoclonal antibody of any one of claims 1 or part thereof and a second monoclonal antibody.

7. A combination according to claim 6, wherein said second monoclonal antibody is selected from antibodies to co–stimulatory receptors on T cells or antigen presenting cells, especially CD40, CD40L, CD80 and CD86.

8. A pharmaceutical composition comprising the antibody of any one of claims 1–3 or the combination of any one of claims 4–7.

9. A pharmaceutical composition according to claim 8, comprising a heat shock protein or peptide fragments of said heat shock protein for the stimulation of type 2 cytokine producing regulatory T cells.

10. A method for treating autoimmune diseases, the method comprising administering to a patient in need of such treatment a therapeutically effective amount of a pharmaceutical composition of claim 8 or 9.

Abstract

Monoclonal antibodies are provided that can bind to the IL12R ß2 chain expressed on the cell surface of human T lymphocytes, where said binding prevents IL12R ß2 chain-mediated STAT4 phosphorylation, and/or said binding prevents the IL12R ß2 chain from the formation of a complex with other membrane-associated proteins, and/or said binding prevents the IL12R ß2 chain from dimerisation to the IL12R ß1 chain.

The antibodies may be combined with autoantigens or with antibodies to co-stimulatory receptors on T cells or antigen presenting cells.

These antibodies and their combinations are also provided as pharmaceutical compositions for the treatment of autoimmune diseases.

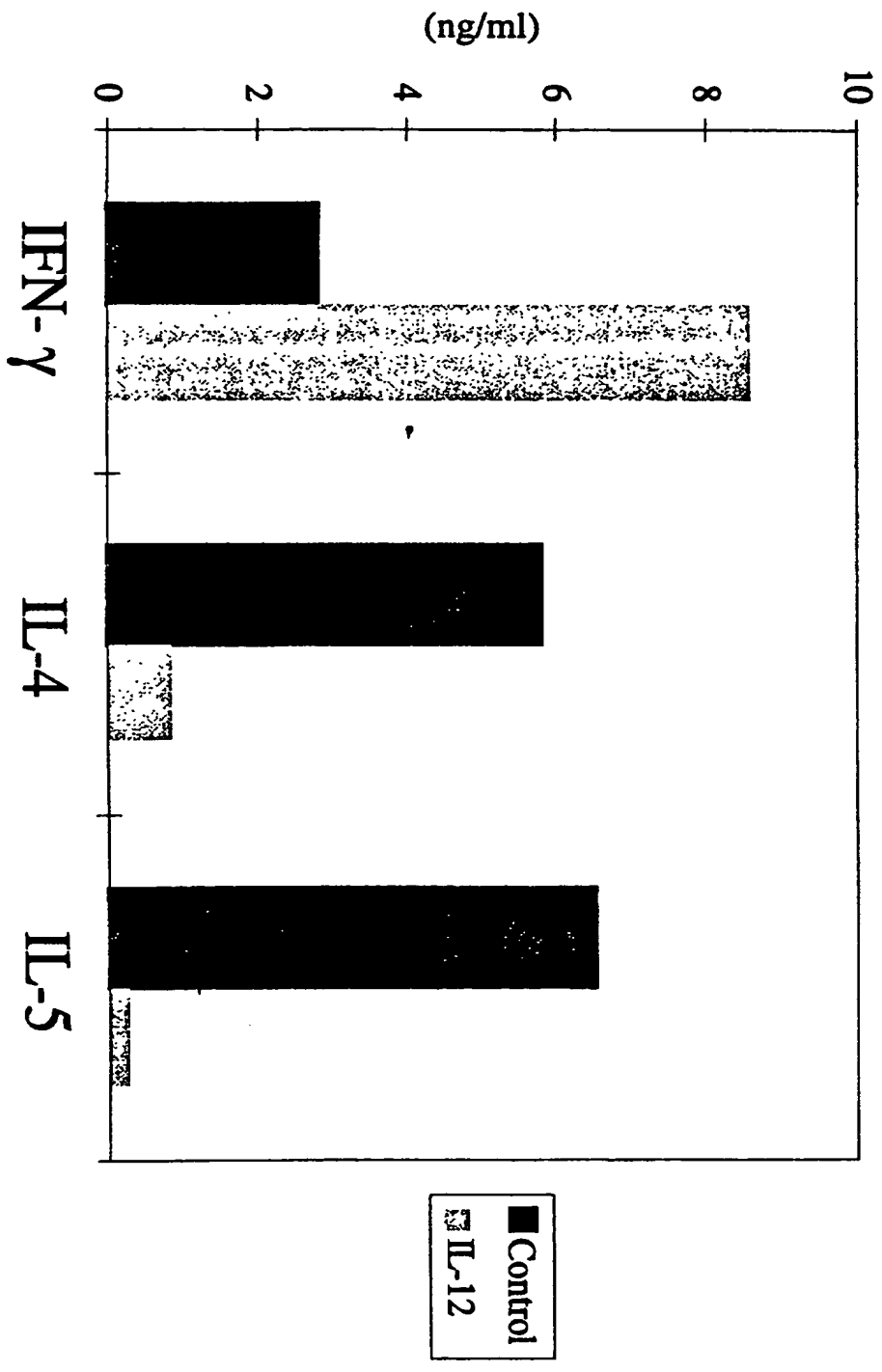

Figure 1